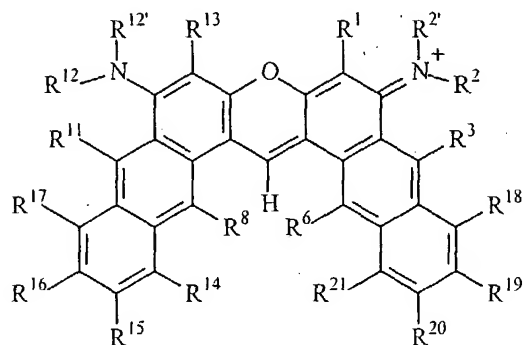
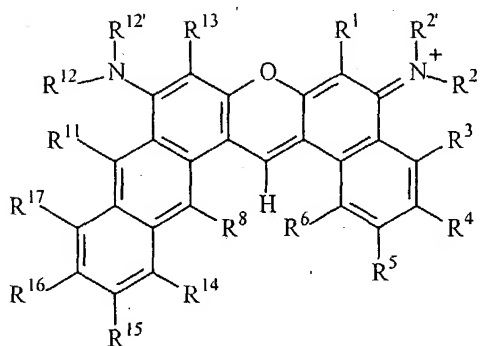
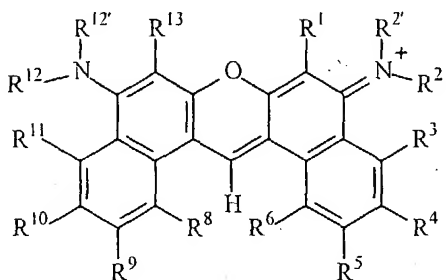


WE CLAIM:

1. Sulfonated diarylrhodamine compounds having the structures:



5 wherein R^2 , $R^{2'}$, R^{12} and $R^{12'}$ is hydrogen, C_1 - C_{12} alkyl, C_1 - C_{12} alkylidyl, phenyl, substituted phenyl, benzyl, substituted benzyl, biphenyl, substituted biphenyl, naphthyl, substituted naphthyl, heterocycle, substituted heterocycle, water-solubilizing group or linking moiety; and

10 R^1 , R^3 , R^4 , R^5 , R^6 , R^8 , R^9 , R^{10} , R^{11} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} , R^{18} , R^{19} , R^{20} , and R^{21} is hydrogen, fluorine, chlorine, C_1 - C_8 alkyl, carboxylate, sulfate, sulfonate, alkylsulfonate, aminomethyl ($-\text{CH}_2\text{NH}_2$), aminoalkyl, 4-dialkylaminopyridinium, hydroxymethyl ($-\text{CH}_2\text{OH}$), methoxy ($-\text{OCH}_3$), hydroxyalkyl ($-\text{ROH}$), thiomethyl ($-\text{CH}_2\text{SH}$), thioalkyl

(-RSH), alkylsulfone (-SO₂R), arylthio (-SAr), arylsulfone (-SO₂Ar), sulfonamide (-SO₂NR₂), alkylsulfoxide (-SOR), arylsulfoxide (-SOAr), amino (-NH₂), ammonium (-NH₃⁺), amido (-CONR₂), nitrile (-CN), C₁-C₈ alkoxy (-OR), phenoxy, phenolic, tolyl, phenyl, aryl, benzyl, heterocycle, phosphonate, phosphate, quaternary amine, sulfate, polyethyleneoxy, water-solubilizing group, or linking moiety;

with the proviso that at least one of R¹, R³, R⁴, R⁵, R⁶, R⁸, R⁹, R¹⁰, R¹¹, R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷, R¹⁸, R¹⁹, R²⁰, and R²¹ is sulfonate.

2. The compound of claim 1 wherein at least one of R², R^{2'}, R¹² and R^{12'} is C₁-C₆ alkylsulfonate or C₄-C₁₀ arylsulfonate.

3. The compound of claim 1 wherein the alkylidiyl, substituted phenyl, substituted benzyl, substituted biphenyl, substituted heterocycle and substituted naphthyl is substituted with sulfonate.

4. The compound of claim 1 wherein the alkylidiyl, substituted phenyl, substituted benzyl, substituted biphenyl, substituted heterocycle and substituted naphthyl is substituted with carboxyl.

5. The compound of claim 1 wherein the linking moiety is azido, monosubstituted primary amine, disubstituted secondary amine, thiol, hydroxyl, halide, epoxide, N-hydroxysuccinimidyl ester, carboxyl, isothiocyanate, sulfonyl chloride, sulfonate ester, silyl halide, chlorotriazinyl, succinimidyl ester, pentafluorophenyl ester, maleimide, haloacetyl, epoxide, alkylhalide, allyl halide, aldehyde, ketone, acylazide, anhydride, iodoacetamide or an activated ester.

6. The compound of claim 1 wherein the water-solubilizing group is carboxylate, sulfonate, phosphonate, phosphate, quaternary amine, sulfate, polyhydroxyl, or water-soluble polymer.

7. The compound of claim 1 wherein the heterocycle is pyrrole, indole, furan, benzofuran, thiophene, benzothiophene, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-quinolyl, 3-quinolyl, 4-quinolyl, 2-imidazole, 4-imidazole, 3-pyrazole, 4-pyrazole, pyridazine, pyrimidine, pyrazine, cinnoline, pthalazine, quinazoline, quinoxaline, 3-(1,2,4-*N*)-triazolyl, 5-(1,2,4-*N*)-triazolyl, 5-tetrazolyl, 4-(1-*O*, 3-*N*)-oxazole, 5-(1-*O*, 3-*N*)-oxazole, 4-(1-*S*, 3-*N*)-thiazole, 5-(1-*S*, 3-*N*)-thiazole, 2-benzoxazole, 2-benzothiazole, 4-(1,2,3-*N*)-benzotriazole, or benzimidazole.

8. The compound of claim 1 wherein R¹, R³, R⁶, R⁸, R¹¹, R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷, R¹⁸, R¹⁹, R²⁰, and R²¹ are hydrogen.

9. The compound of claim 1 comprising a first bridging group which when taken together with the C-12-bonded nitrogen and the C-12 and C-13 carbons forms a first ring structure having from 4 to 7 members; and/or

a second bridging group which when taken together with the C-2-bonded-nitrogen
5 and the C-1 and C-2 carbons forms a second ring structure having from 4 to 7 members.

10. The compound of claim 9 wherein one or both of the first and second ring structures has five members.

11. The compound of claim 10 wherein the five membered ring structure includes one gem disubstituted carbon.

10 12. The compound of claim 11 wherein the gem substituents are (C₁-C₈) alkyl.

13. The compound of claim 11 wherein the gem substituents are methyl.

14. The compound of claim 10 wherein the five membered ring is substituted with linking moiety or water-solubilizing group.

15 15. The compound of claim 1 comprising a third bridging group which when taken together with the C-12-bonded nitrogen and the C-11 and C-12 carbons forms a third ring structure having from 5 to 7 members; and/or

a fourth bridging group which when taken together with the C-2-bonded nitrogen and the C-2 and C-3 carbons forms a fourth ring structure having from 5 to 7 members.

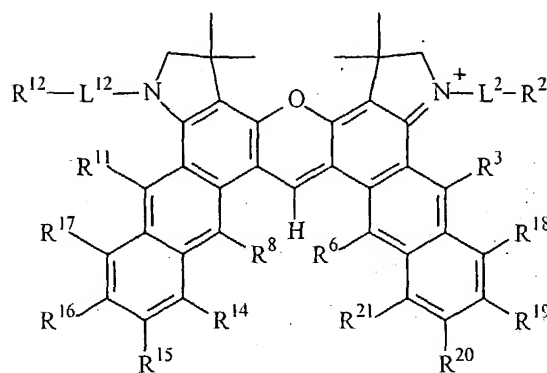
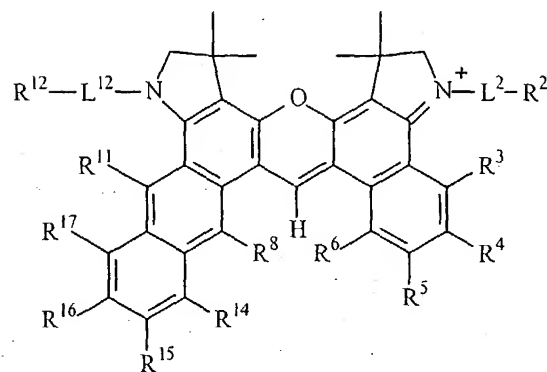
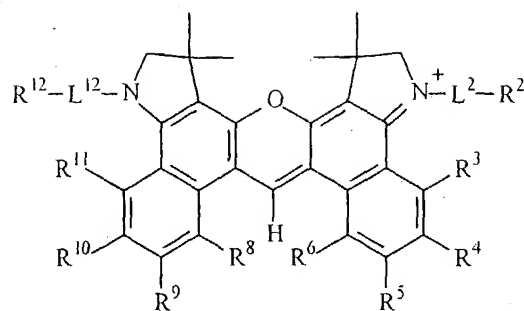
20 16. The compound of claim 15 wherein one or both of the third and fourth ring structures has six members.

17. The compound of claim 16 wherein the six membered ring structure includes one gem disubstituted carbon.

18. The compound of claim 17 wherein the gem substituents are (C₁-C₈) alkyl.

25 19. The compound of claim 18 wherein the gem substituents are methyl.

20. The compound of claim 1 having the structures:



wherein L^2 and L^{12} are linkers selected from the group consisting of alkylidyl, substituted phenyl, substituted benzyl, substituted biphenyl, substituted heterocycle and substituted naphthyl.

21. The compound of claim 1 comprising a fused aromatic ring bonded across the C-3 and C-4 carbons, the C-4 and C-5 carbons, the C-9 and C-10 carbons, or the C-10 and C-11 carbons, including substituted forms thereof.

22. The compound of claim 1 comprising a fused aromatic ring bonded across the C-3 and C-4 carbons and the C-10 and C-11 carbons, including substituted forms thereof.

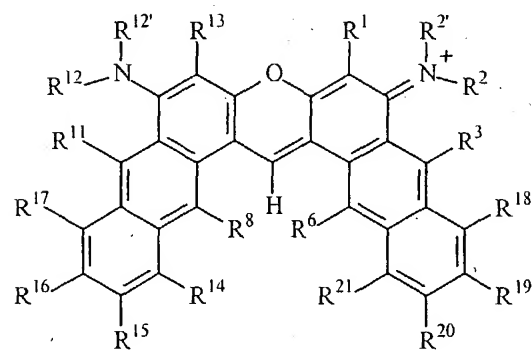
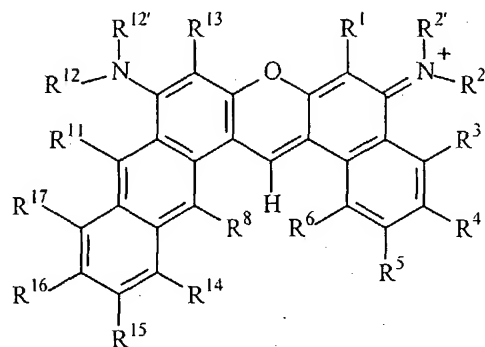
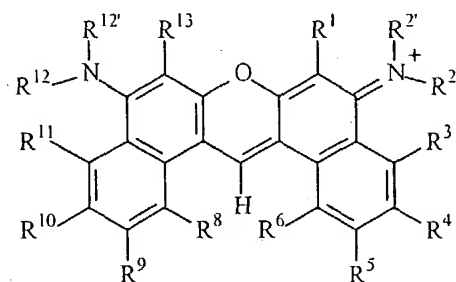
23. An energy-transfer dye comprising:
a donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response thereto;

an acceptor dye capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response; and

a linker for linking the donor dye and the acceptor dye;

wherein at least one of the donor dye and acceptor dye is a sulfonated

5 diarylrhodamine compound having one of the structures:



including nitrogen- and aryl-substituted forms thereof, with the proviso that at least one of $R^1, R^3, R^4, R^5, R^6, R^8, R^9, R^{10}, R^{11}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}$, and R^{21} is sulfonate.

10 24. The energy-transfer dye of claim 23 wherein the acceptor dye is a sulfonated diarylrhodamine compound and the donor dye is a cyanine, a

phthalocyanine, a squaraine, a bodipy, a benzophenoxazine, a fluorescein, a dibenzorhodamine, or a rhodamine dye.

25. The energy-transfer dye of claim 24 wherein the acceptor dye is a sulfonated diarylrhodamine compound and the donor dye is a fluorescein or a rhodamine linked to the acceptor dye and to a polynucleotide.

26. The energy-transfer dye of claim 25 wherein the donor dye is linked to the 5'-terminus of the polynucleotide.

27. The energy-transfer dye of claim 25 wherein the donor dye is linked to the 3'-terminus of the polynucleotide.

28. The energy-transfer dye of claim 25 wherein the donor dye is linked to a nucleobase of the polynucleotide, wherein if the nucleobase is a purine, the linker is attached at the 8-position; if the nucleobase is a 7-deazapurine, the linker is attached at the 7-position or 8-position and; if the nucleobase is a pyrimidine, the linker is attached at the 5-position.

29. The energy-transfer dye of claim 23 wherein the linker has the structures:



wherein

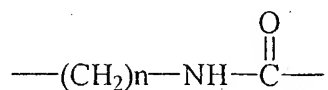
Z is selected from the group consisting of NH, S and O;

R²¹ is a C₁-C₁₂ alkyl attached to the donor dye;

R²² is a substituent selected from the group consisting of a C₁-C₁₂ alkylidyl, a five and six membered ring having at least one unsaturated bond and a fused ring structure which is attached to the carbonyl carbon; and

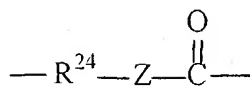
R²³ includes a functional group which attaches the linker to the acceptor dye.

30. The compound of claim 29 wherein the linker has the structure:



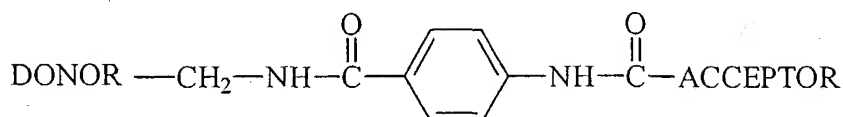
and n ranges from 2 to 10.

31. The energy-transfer dye of claim 29 wherein R^{23} has the structure

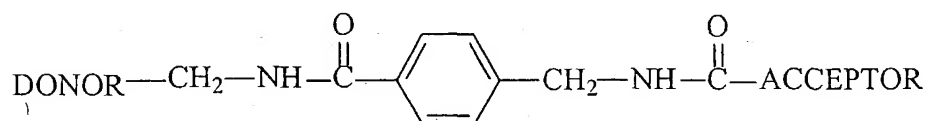


wherein R^{24} is a C_1-C_{12} alkyl.

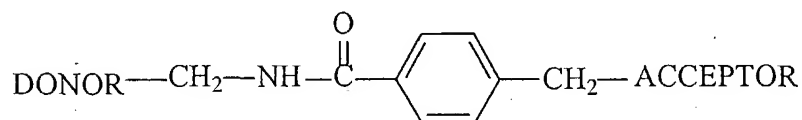
32. The energy-transfer dye of claim 23 wherein the linker has the structure



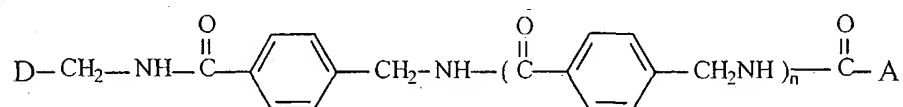
33. The energy-transfer dye of claim 23 wherein the linker has the structure



34. The energy-transfer dye of claim 23 wherein the linker has the structure



35. The energy-transfer dye of claim 23 in which the linker has the structure:



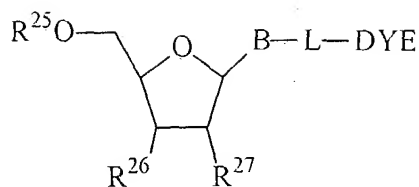
wherein D is a donor dye, A is an acceptor dye and n is 1 or 2.

36. The energy-transfer dye of claim 23 wherein the linker is attached at R^2 ,

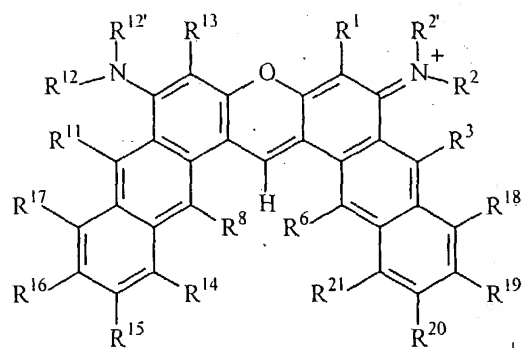
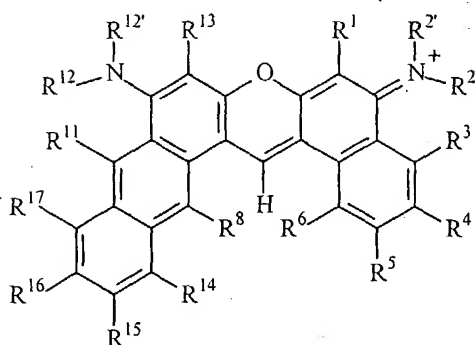
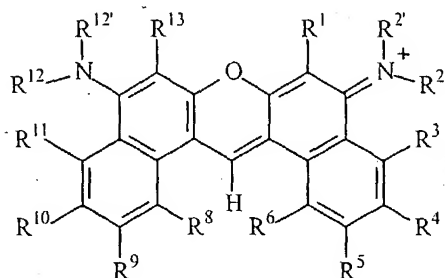
$R^{2'}$, R^{12} or $R^{12'}$ of the sulfonated diarylrhodamine compound.

37. The energy-transfer dye of claim 36 wherein a linker to the donor or the acceptor is attached to R^2 or $R^{2'}$, and a linker to a polynucleotide is attached to R^{12} or $R^{12'}$.

38. A labelled nucleoside or nucleotide having the formula:



wherein DYE is a sulfonated diarylrhodamine compound having one of the structures:



- 5 including nitrogen- and aryl-substituted forms thereof, with the proviso that at least one of R¹, R³, R⁴, R⁵, R⁶, R⁸, R⁹, R¹⁰, R¹¹, R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷, R¹⁸, R¹⁹, R²⁰, and R²¹ is sulfonate, or

DYE is an energy-transfer dye comprising a donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response thereto; an

- 10 acceptor dye capable of absorbing the excitation energy emitted by the donor dye and

fluorescing at a second wavelength in response; and a linker for linking the donor dye and the acceptor dye; wherein at least one of the donor dye and acceptor dye is a said sulfonated diarylrhodamine compound;

B is a nucleobase;

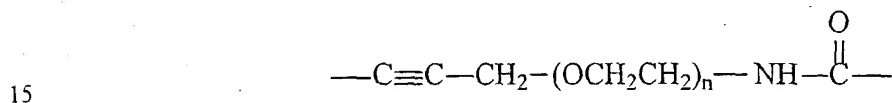
5 L is a linker;

R²⁵ is H, monophosphate, diphosphate, triphosphate, thiophosphate, or phosphate analog; and

10 R²⁶ and R²⁷, when taken alone, are each independently H, HO, F, and a moiety which blocks polymerase-mediated target-directed polymerization, or when taken together form 2'-3'-didehydroribose.

39. The labelled nucleoside or nucleotide of claim 38 wherein B is selected from the group consisting of uracil, thymine, cytosine, adenine, 7-deazaadenine, guanine, and 7-deazaguanosine.

40. The labelled nucleoside or nucleotide of claim 38 in which L is:

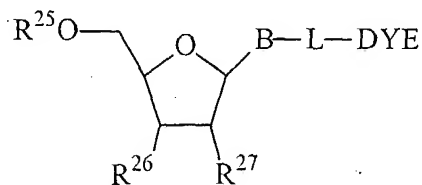


wherein n is 0, 1, or 2.

41. The labelled nucleoside or nucleotide of claim 38 which is enzymatically incorporatable.

42. The labelled nucleoside or nucleotide of claim 38 which is a terminator.

20 43. The labelled nucleoside or nucleotide of claim 42 which has the structure:

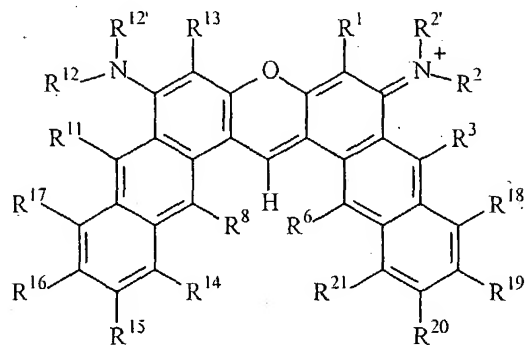
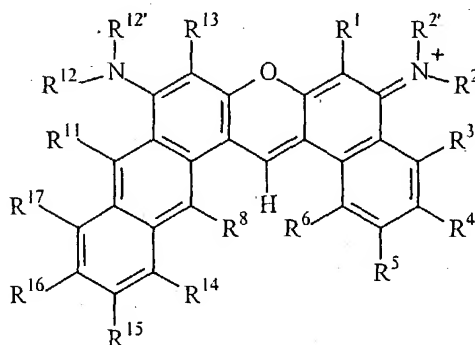
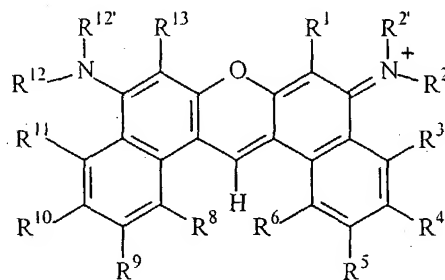


wherein R²⁶ and R²⁷, when taken alone, are each independently H, F, and a moiety which blocks polymerase-mediated target-directed polymerization, or when taken together form 2'-3'-didehydroribose.

44. The labelled nucleoside or nucleotide of claim 38 which is enzymatically extendable.

45. A labelled polynucleotide comprising a polynucleotide covalently attached to a label, wherein the label is a sulfonated diarylrhodamine compound having

5 one of the structures:



including nitrogen- and aryl-substituted forms thereof, with the proviso that at least one of $R^1, R^3, R^4, R^5, R^6, R^8, R^9, R^{10}, R^{11}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}$, and R^{21} is sulfonate, or

10 an energy-transfer dye comprising a donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response thereto; an acceptor dye capable of absorbing the excitation energy emitted by the donor dye and fluorescing at

R^{27} is H, OH, halide, azide, amine, alkylamine, C_1-C_6 alkyl, allyl, C_1-C_6 alkoxy, OCH_3 , or $OCH_2CH=CH_2$; and

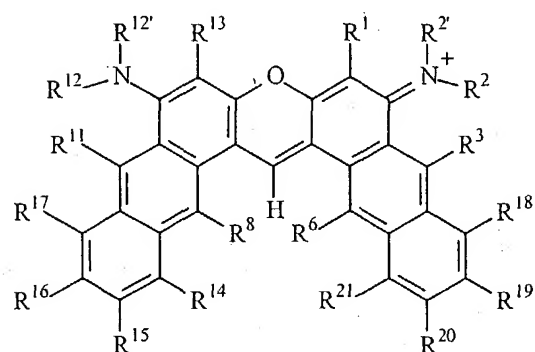
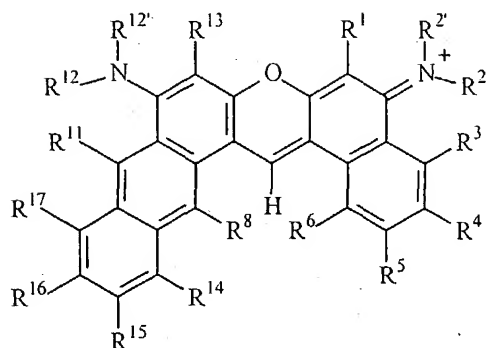
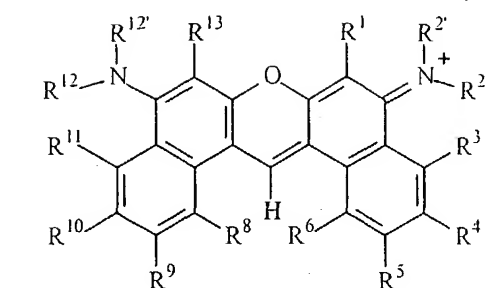
R^{28} is internucleotide phosphodiester or internucleotide analog;

wherein the polynucleotide comprises 2 to 1000 nucleotides.

5 49. The labelled polynucleotide of claim 48 wherein B is selected from the group consisting of uracil, thymine, cytosine, adenine, 7-deazaadenine, guanine, and 7-deazaguanosine.

50. The labelled polynucleotide of claim 48 in which L is C_1-C_{12} alkylidyl or $-(CH_2CH_2O)_n-$, where n is 1 to 100.

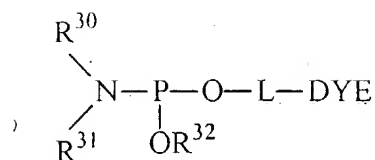
10 51. A labelled polypeptide comprising a polypeptide covalently attached to a sulfonated diarylrhodamine compound having one of the structures:



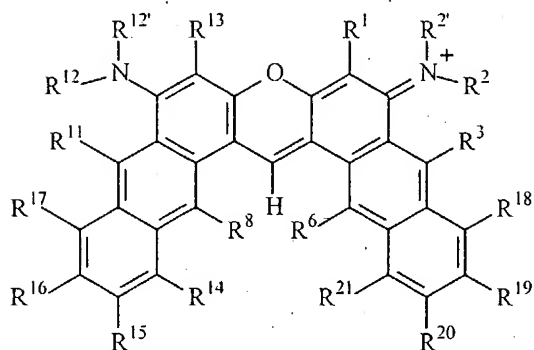
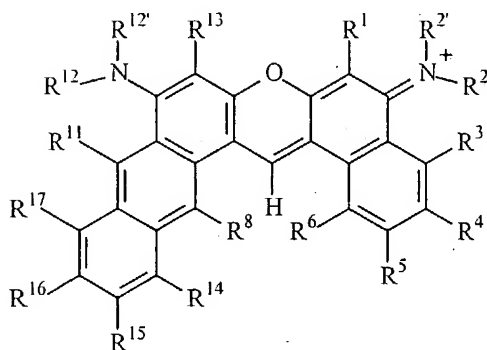
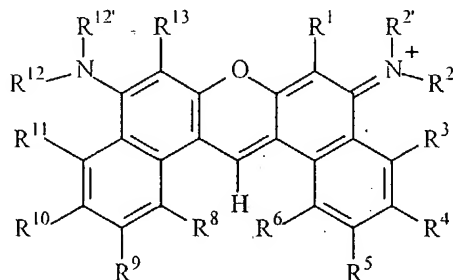
including nitrogen- and aryl-substituted forms thereof, with the proviso that at least one of $R^1, R^3, R^4, R^5, R^6, R^8, R^9, R^{10}, R^{11}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}$, and R^{21} is sulfonate, or

- 5 the polypeptide covalently attached to an energy-transfer dye comprising a donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response thereto; an acceptor dye capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response; and a linker for linking the donor dye and the acceptor dye; wherein at least one of the donor dye and acceptor dye is a
- 10 said sulfonated diaryl rhodamine compound.

52. A phosphoramidite compound having the formula:



wherein DYE is a sulfonated diarylrhodamine compound having one of the structures:



- 5 including nitrogen- and aryl-substituted forms thereof, with the proviso that at least one of R¹, R³, R⁴, R⁵, R⁶, R⁸, R⁹, R¹⁰, R¹¹, R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷, R¹⁸, R¹⁹, R²⁰, and R²¹ is sulfonate, or

DYE is an energy-transfer dye comprising a donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response thereto; an
10 acceptor dye capable of absorbing the excitation energy emitted by the donor dye and

fluorescing at a second wavelength in response; and a linker for linking the donor dye and the acceptor dye; wherein at least one of the donor dye and acceptor dye is a said sulfonated diarylrhodamine compound;

L is a linker;

R^{30} and R^{31} taken separately are selected from the group consisting of C_1 - C_{12} alkyl, C_1 - C_{12} cycloalkyl, and aryl; or R^{30} and R^{31} taken together with the nitrogen atom form a saturated nitrogen heterocycle; and

R^{32} is a phosphite ester protecting group.

53. The phosphoramidite compound of claim 52 wherein R^{32} is selected from the group consisting of methyl, 2-cyanoethyl, and 2-(4-nitrophenyl)ethyl.

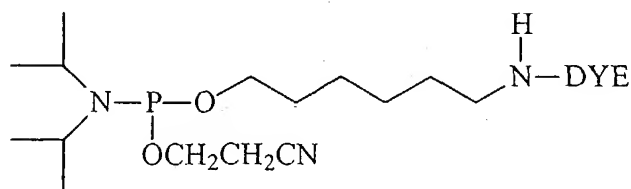
54. The phosphoramidite compound of claim 52 wherein R^{30} and R^{31} are each isopropyl.

55. The phosphoramidite compound of claim 52 wherein R^{30} and R^{31} taken together is morpholino.

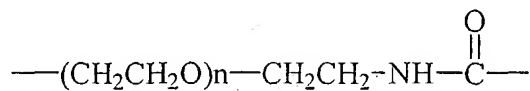
56. The phosphoramidite compound of claim 52 wherein L is C_1 - C_{12} alkyldiyl.

57. The phosphoramidite compound of claim 52 wherein L is attached at R^2 , R^2 , R^{12} or R^{12} of the DYE.

58. The phosphoramidite compound of claim 57 having the structure:



59. The phosphoramidite compound of claim 57 wherein L is



and n ranges from 1 to 10.

60. A method of forming a labelled substrate comprising the step of reacting a substrate selected from the group consisting of a polynucleotide, a nucleotide, a

nucleoside, a polypeptide, a carbohydrate, a ligand, a particle, and a surface, with the linking moiety of the compound of claim 1 or the energy-transfer dye of claim 23, whereby a labelled substrate is formed.

61. The method of claim 60 wherein the linking moiety is N-hydroxysuccinimide.

62. The method of claim 60 wherein the linking moiety is a phosphoramidite.

63. The method of claim 60 wherein the particle is a nanoparticle, a microsphere, a bead, or a liposome.

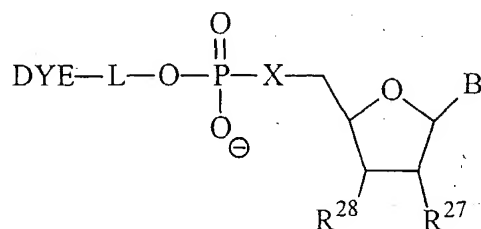
64. The method of claim 60 wherein the surface is glass.

65. A method of synthesizing a labelled polynucleotide comprising the step of coupling the phosphoramidite compound of claim 52 to a polynucleotide, wherein the polynucleotide is bound to a solid support, whereby a labelled polynucleotide is formed.

66. A method of generating a labelled primer extension product, comprising the step of extending a primer-target hybrid with an enzymatically-incorporatable nucleotide, wherein said primer or said nucleotide is labelled with a compound according to claim 1 or an energy-transfer compound of claim 23, whereby the primer is extended.

67. The method of claim 66 wherein the nucleotide is enzymatically-extendable.

68. The method of claim 66 wherein the primer is a labelled polynucleotide comprising the formula:



wherein DYE is a sulfonated diarylrhodamine compound or an energy-transfer dye;

B is a nucleobase selected from the group consisting of uracil, thymine, cytosine, adenine, 7-deazaadenine, guanine, and 7-deazaguanosine;

X is O, NH, or S;

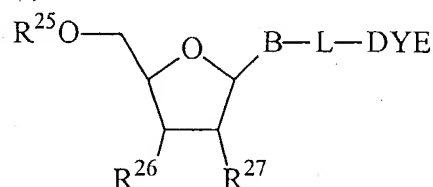
L is a linker;

R²⁷ is H, OH, halide, azide, amine, alkylamine, C₁-C₆ alkyl, allyl, C₁-C₆ alkoxy, OCH₃, or OCH₂CH=CH₂; and

R²⁸ is internucleotide phosphodiester or internucleotide analog;

wherein the polynucleotide comprises 2 to 100 nucleotides.

69. The method of claim 66 wherein the enzymatically-incorporatable nucleotide is a labelled nucleoside or nucleotide having the formula:



wherein DYE is a sulfonated diarylrhodamine compound or an energy-transfer dye;

B is a nucleobase selected from the group consisting of uracil, thymine, cytosine, adenine, 7-deazaadenine, guanine, and 7-deazaguanosine;

L is a linker;

R²⁵ is H, monophosphate, diphosphate, triphosphate, thiophosphate, or phosphate analog; and

R²⁶ and R²⁷, when taken alone, are each independently H, HO, and F.

70. The method of claim 66 further comprising a terminator nucleotide.

71. The method of claim 69 wherein R²⁶ and R²⁷, when taken alone, are each independently H, F, a moiety which blocks polymerase-mediated target-directed primer extension, or when taken together form 2'-3'-didehydroribose.

72. A method of polynucleotide sequencing comprising the steps of:
forming a mixture of a first, a second, a third, and a fourth class of polynucleotides such that:

each polynucleotide in the first class includes a 3'-terminal dideoxyadenosine and is labelled with a first dye;

each polynucleotide in the second class includes a 3'-terminal dideoxycytidine and is labelled with a second dye;

each polynucleotide in the third class includes a 3'-terminal dideoxyguanosine and is labelled with a third dye; and

5 each polynucleotide in the fourth class includes a 3'-terminal dideoxythymidine and is labelled with a fourth dye;

wherein one or more of the first, second, third, or fourth dyes is a sulfonated diarylrhodamine compound according to claim 1 or an energy-transfer dye of claim 23, the other dyes are spectrally resolvable from each other; and

10 separating the polynucleotides by electrophoresis.

73. The method of claim 72 further comprising the step of detecting the separated polynucleotides by fluorescence detection.

74. The method of claim 72 further comprising the step of identifying the 3'-terminal nucleotide of the polynucleotides by the fluorescence spectrum of the dyes.

15 75. A method of oligonucleotide ligation comprising the steps of annealing two probes to a target sequence and forming a phosphodiester bond between the 5' terminus of one probe and the 3' terminus of the other probe;

wherein one or both probes are labelled with a compound according to claim 1 or an energy-transfer dye of claim 23.

20 76. A method of fragment analysis comprising the steps of separating labelled polynucleotide fragments by a size-dependent separation process, and detecting the separated labelled polynucleotide fragments subsequent to the separation process;

wherein the fragments are labelled with a compound of claim 1 or an energy-transfer dye of claim 23.

25 77. The method of claim 76 wherein the fragments are labelled with a mobility-modifying label.

78. The method of claim 76 wherein the fragments are formed by ligation.

79. The method of claim 76 wherein the size-dependent separation process is electrophoresis and the labelled polynucleotide fragments are detected by fluorescence.

80. A method of amplification comprising the steps of annealing two or more primers to a target polynucleotide and extending the primers by a polymerase and a mixture of enzymatically-extendable nucleotides;

wherein at least one of the primers is a labelled polynucleotide according to claim

45:

81. A method of amplification comprising the steps of annealing two or more primers to a target polynucleotide and extending the primers by a polymerase and a mixture of enzymatically-extendable nucleotides;

wherein at least one of the nucleotides is a labelled nucleotide according to claim 38.

82. A method of amplification comprising the steps of annealing two or more primers and a fluorescent dye-quencher probe to a target nucleic acid and extending the primers by polymerase and a mixture of enzymatically-extendable nucleotides;

wherein the probe is a labelled polynucleotide according to claim 45.

83. A method of hybridization comprising the steps of annealing a target polynucleotide to an immobilized probe and detecting the fluorescence from the target-probe complex;

wherein the target polynucleotide is a labelled polynucleotide according to claim 45 and the immobilized probe is covalently attached to a planar surface.

84. The method of claim 83 wherein the planar surface is an addressable array.

85. A kit for labelling a polynucleotide, comprising a sulfonated diarylrhodamine compound including a linking moiety according to claim 5 and a polynucleotide.

86. A kit for labelling a polynucleotide, comprising a energy-transfer dye according to claim 23 and a polynucleotide.

87. A kit for labelling a polynucleotide, comprising a phosphoramidite compound according to claim 52 and a polynucleotide.

88. A kit for generating a labelled primer extension product, comprising one or more enzymatically-incorporatable nucleotides and a primer, wherein said primer is a labelled polynucleotide according to claim 45.

89. A kit for generating a labelled primer extension product, comprising one or more enzymatically-incorporatable nucleotides and a primer, wherein at least one nucleotide is a labelled nucleotide according to claim 38.

90. The kit of claim 89 wherein the labelled nucleotide is a terminator.

5 91. The kit of claim 90 which comprises four different terminators, one which terminates at a target A, one which terminates at a target G, one which terminates at a target C and one which terminates at a target T or U.

92. A method of synthesis of sulfonated aminonaphthalene and aminoanthracene compounds comprising the steps of:

10 protecting the amino group with a protecting group reagent;
reacting the protected amino compound with chlorosulfonic acid; and
removing the amino protecting group.